

V3 loop sequence data of HIV-1 patient isolates (PI)

	CTRPNNNTRKSI	HIGPGRAFYATGDIIGDIRQAHC
PI-903	-----G-----	STN-----A-----S-----
PI-951	-----H-----	N-----W-T-----
PI-918	-----S-----	-----E-----
PI-970	-----S-----	-----E-----
PI-990	-----R-----	P-----T-----V-----
PI-991	-----P-----	A-----E-----N-----
PI-952	-I-----R-----	TL-----VL-T-----E-----K-----
PI-932	-I--H-TVTDR--	-----S-HT-RKIK-----
PI-910	----SIQK-R-V.R----	-----S-I-ARAAT-----K-Q-----
PI-911	----SIQK-R-V.R----	-----S-I--RAAT-----K-Q-----
PI-930	----YR-AKHR-M-----	-----NVKGNIK:-----

Fig. 2a

2/6

Amino acid sequences of the NL4-3 V2 loop and NL4-3 V3 loop. The regions which are preferably to be varied are underlined.

NL4-3 V2 Loop

GEIKNCSFNISTSIRDKVOKEYAFFYKLDIVPIDNTSYRLISCNTSVITQA

NL4-3 V3 Loop

VQLNTSVEINCTRPNNNTRKSIRIQRGPCRAFVTIGKIGNMRQAHCNISRAKWNATLKQ

Fig. 3

4/6

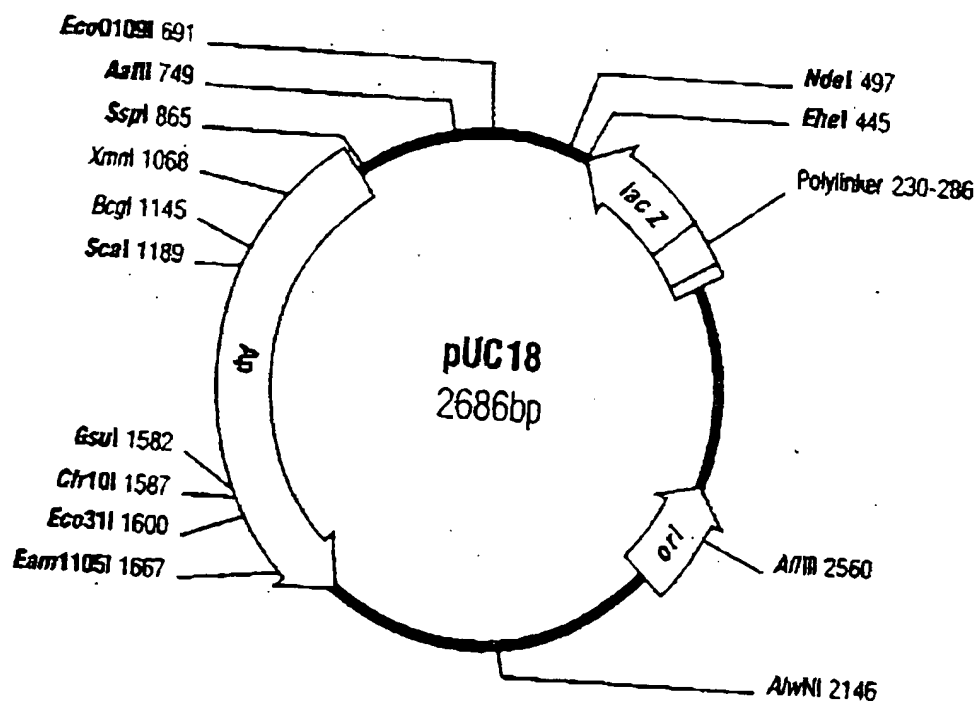


Fig. 4

5/6

Schematic representation of the process for the preparation of the mixture of gp 120-expressing plasmid vectors.

- 1.) Preparation of the degenerated DNA fragments for e.g. the V3 loop
 - a.) Synthesis of single-stranded DNA
 - b.) Hybridization of two complementary oligonucleotides.
- 2.) Cloning of the V3 loop DNA fragments into pUC18 delta env
- 3.) Cloning of the env gene into the gp 120 expression vector pBSCenvATG

V3 loop fragments with degenerated sequence e.g.:

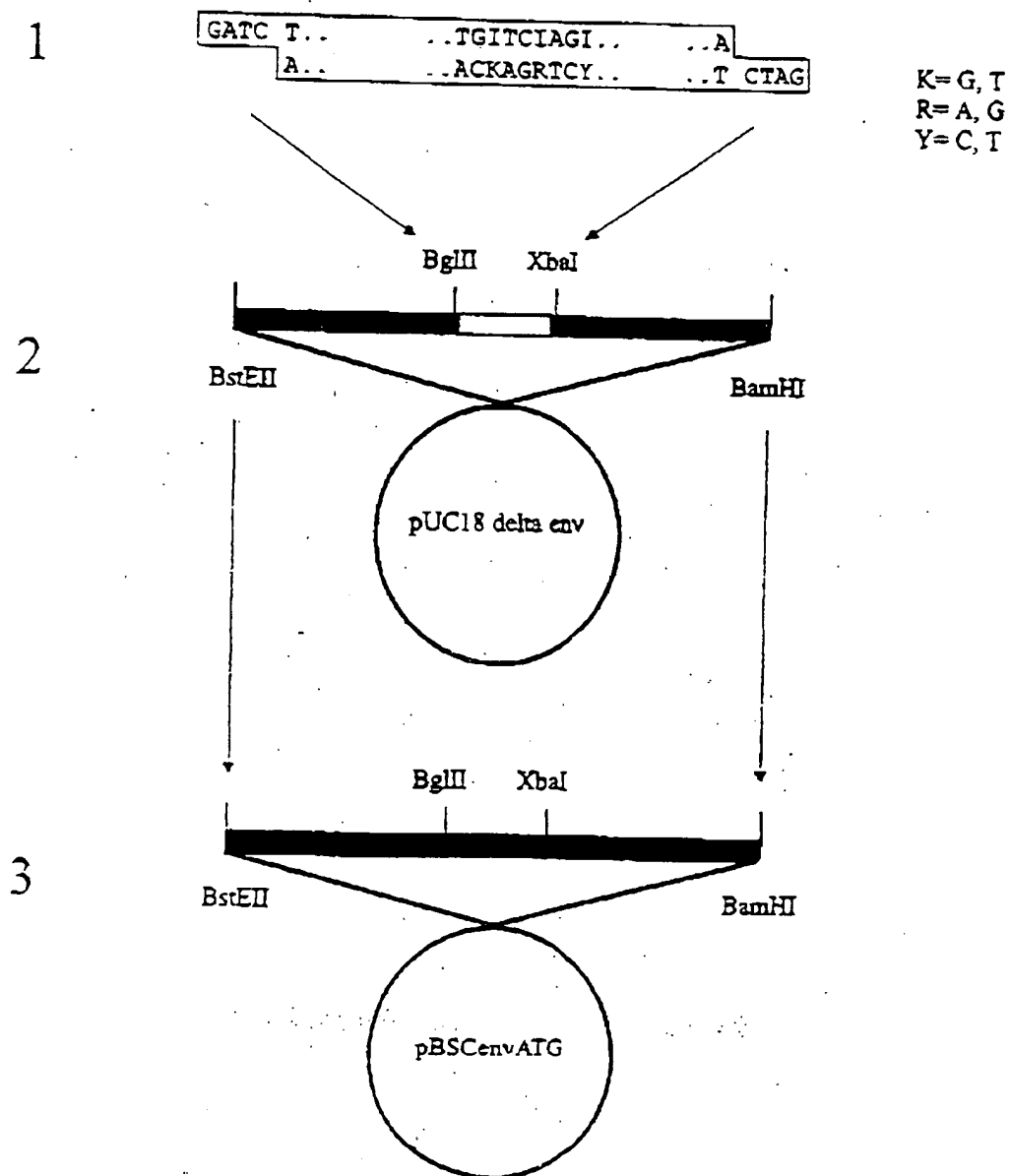


Fig. 5

6/6

Heterogeneity of the vaccine using the V3 loop as an example

V3 loop sequence:

CTRPNNNTKRSIHIGPGRAFYTGTGDIIGDIRQAHC

G	P	HA	KK	E
R		RN		
		ET		
		E		
		A		

Degeneration at protein level:

$3 \times 2 \times 2 \times 2 \times 4 \times 6 \times 2 = 1152$ variants

Ser	His	Tyr	Thr	Gly	Asp	Asp
Gly	Pro	His	Ala	Lys	Lys	Glu
Arg				Arg	Asn	
				Glu	Thr	
				Glu		
				Ala		

Degenerated DNA sequence of the respective variable amino acid positions:

AGA CCT TAT ACT GGG GAG GAC
G T A C G AA ACC G = 2048 variants

AGA	CCT	TAT	ACT	GGG	GAG	GAC
AGT	CAT	CAT	GCT	GAG	GAC	GAG
GGT				AGG	GCG	
GGA				AAG	GCC	
				AAG		
				AAC		
				ACG		
				ACC		

Variability at protein level derived from the degenerated DNA sequence:

Arg	Pro	Tyr	Thr	Gly	Glu	Asp
Ser	His	His	Ala	Glu	Asp	Glu
Gly				Arg	Ala	
Gly				Lys	Ala	
				Lys		
				Asn		
				Thr		
				Thr		